52*

TNF-alpha recruits CFTR into lipid rafts in MDCK cells

S. Huang, T. Dudez, B. Stanton, G. Lukacs, S. Suter, M. Chanson
Geneva University Hospitals, Geneva, Switzerland

We have shown that CFTR is required for activation of the tyrosine kinase c-Src in airway epithelial cell lines exposed to TNF-alpha. To understand the molecular mechanisms that link CFTR to c-Src, we investigated whether CFTR is present in cholesterol-enriched membrane microdomain lipid rafts. Lipid rafts, which serve as platforms for efficient signal transduction in a number of cell systems, have been shown to contain c-Src. Lipid rafts were isolated from MDCKI or MDCKII cells expressing GFP-tagged wild-type CFTR by density gradient ultracentrifugation and Western blots. CFTR was detected in non-soluble fractions using antibodies against CFTR or GFP. Quality of the preparation was confirmed by the detection of lipid raft markers, such as caveolin-1, flottilin-1 as well as the tyrosine kinases c-Src and c-Yes. The amount of CFTR in lipid rafts, which represented about 10% of total CFTR, was reduced by extraction of cell surface cholesterol with cyclodextrin and increased by depolymerization of actin filaments with latrunculin. Interestingly, exposure of MDCKI or MDCKII cells to TNF-alpha increased the proportion of c-Src and CFTR present in lipid rafts. CFTR, however, was not recovered in membrane microdomains from MDCK cells expressing a truncated CFTR lacking its four last c-terminal amino acids (DTRL), exposed or not to TNF-alpha. The data indicate that in two expression cell systems, CFTR-containing lipid rafts are dynamic structures that might be stabilized by the cytoskeleton via PDZ-binding domain's protein interaction, and that this interaction can be modulated by pro-inflammatory mediators.

Supported by the Swiss National Science Foundation and Vaincre la Mucoviscidose.

53

Inhibition by TNF-alpha and IL-4 of cationic lipid mediated gene transfer in cystic fibrosis tracheal gland cells

S. Bastonero, M. Gargouri, S. Ortiou, J.L. Guéant, M.D. Merten
Laboratoire de Pathologie Cellulaire et Moléculaire en Nutrition. Equipe INSERM U 724, Faculté de médecine, PB 184, 54505 Vandoeuvre-lès-Nancy, France

Background: In vivo, tracheal gland serous cells highly express the Cystic Fibrosis Transmembrane conductance Regulator (*cftr*) gene. This gene is mutated in the letal monogenic disease Cystic Fibrosis (CF). Clinical trials in which the *cftr* cDNA was delivered to the respiratory epithelia of CF patients have resulted in modest, transient gene expression and yet no improvement of their clinical status.
Methods and results: As CF is characterized by mucus inspissation, airway infection, and severe inflammation, we tested the hypothesis that inflammation and especially two cytokines involved in the Th1/Th2 inflammatory response, Interleukin 4 (IL-4) and TNFα could inhibit gene transfer efficiency using a model of human CF tracheal gland serous cells (CF-KM4) and Lipofectamine reagent as a vector. The specific secretory defects of CF-KM4 cells were corrected by lipofectamine-mediated *cftr* gene transfer. However, this was altered when cells were pre-treated with IL-4 and TNFα. Expression of luciferase reporter gene by IL-4 and TNFα pre-treated CF-KM4 cells was measured by activity and real-time RT-PCR. Both cytokines induced similar and synergistic inhibition of transgene expression and activity. This cytokine-mediated inhibition could be prevented by anti-inflammatory agents such as glucocorticoids but not by non steroidal (NSAI) agents.
Conclusions: This data suggests that an inflammatory context generated by IL-4 and TNFα can inhibit *cftr* gene transfer in CF tracheal gland serous cells and that glucocorticoids may have a protecting action.

54

Immunological detection of human CFTR after gene transfer to the lung

A. Wilson, H. Davidson, S.T. Cooper, G. McLachlan, D.J. Porteous, A.C. Boyd
Medical Sciences (Medical Genetics), University of Edinburgh, EH4 2XU, UK and UK Cystic Fibrosis Gene Therapy Consortium

Our aim is to use the sheep as a model to refine immunohistochemical (IHC) assays to detect transgenic human CFTR (hCFTR) in patients treated by gene therapy. Nasal brushings (NBs) from F508del/F508del patients have fewer cells with apically localised CFTR compared to normal brushings. We have found that inter-subject variation is so high that measuring the efficacy of CF gene therapy by simply counting cells with apical hCFTR may be problematic. Here we explore another approach.
NB cells from humans and sheep were harvested, fixed and cytospun onto glass slides. Sections were cut from frozen samples of sheep lung and airways, then fixed in formaldehyde and sucrose before IHC.
Human NB cells were examined using dilutions of G449 (an antibody which does not detect ovine CFTR): at 1:600 the apical signal was found to be just visible. Sections from airways of sheep aerosolised using a polymer/hCFTR plasmid complex were doubly labelled with G449 at 1:600, and the antibody MATG1061 (which detects both CFTRs) at various dilutions. At its usual dilution (1:100), MATG1061 labelled many cells, but was found to label only G449-positive cells at 1:250.
The monospecificity of G449 allowed us to identify hCFTR-transfected cells in sheep. With this information, we determined the dilution at which MATG1061 detects cells expressing hCFTR, but not the ovine CFTR background. This method permits the optimisation of dilution conditions for CFTR antibodies that may then be used to specifically detect transgenic hCFTR expression in the airways of patients treated with CF gene therapy.
We thank the CF Trust for support, and Hugo de Jonge, Angus Nairn and Transgène for generous gifts of antibody.

55

Tight junction regulation and AAV-2 mediated gene transfer in a cystic fibrosis airway epithelial cell line

N. Makeeva, O. Forsberg, N. Welsh, G.M. Roomans
Department of Medical Cell Biology, University of Uppsala, Sweden

Virus-mediated gene therapy is one possible approach to treatment of cystic fibrosis. Adeno-associated viruses (AAV) may be relatively suitable vectors. However, airway epithelium is resistant to infection by AAV-2 when the virus is applied to the luminal surface of the cells. A possible strategy for enhancing gene transfer is to modify the paracellular permeability, thereby permitting the diffusion of vectors to the basolateral surface, where uptake receptors are expressed.
The effect of different substances on paracellular permeability in a monolayer culture of CF airway epithelial cells (CFBE41o- cells) was investigated by measuring the transepithelial electrical resistance (TEER). CFBE41o- cells reached a stable TEER of about 800 Ωcm^2 after 9-13 days in culture. Sodium caprate (C10) very rapidly reduced TEER, and with 20 or 30 mM C10 levels of 100 Ωcm^2 and below were reached after 1-2 minutes. With EGTA about one hour is needed to reduce TEER to these levels. Also high concentrations of NaCl (202.5 mM) and D-mannitol (405 mM) reduce TEER but not to the same extent as C10.
AAV-2 transfer to the epithelial cells was determined using the Lac-Z gene as a reporter gene. Treatment with C10 resulted in a considerable and concentration-dependent enhancement of AAV-2-LacZ gene transfer. At 20 mM C10 60% of the cells, and at 30 mM C10 90% of the cells were transfected. EGTA, D-mannitol and NaCl showed very little efficiency in enhancing gene transfer. One major concern was, however, the induction of cell death by the different TJ regulating agents.
The results indicate that C10 may be a better agent for enhancing gene transfer than EGTA, D-mannitol and NaCl and suggest that AAV-2 can be a suitable vector for gene transfer through the basolateral membrane.